Effects of F-strain *Mycoplasma gallisepticum* Inoculation at Twelve Weeks of Age on Egg Yolk Composition in Commercial Egg Laying Hens[1,2]

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ABSTRACT In two trials, the effects of F-strain *Mycoplasma gallisepticum* (FMG) on the contents of egg yolks from commercial Single Comb White Leghorn laying hens were investigated over a production cycle. Ten hens were assigned to each of 8 (trial 1) or 16 (trial 2) negative pressure fiberglass biological isolation units. Birds in half of the total units served as sham-inoculated controls, and those in the other half were inoculated with FMG at 12 wk of age. Eggs were collected and yolks were harvested at various times during the prepeak, peak, and postpeak periods of both trials for constituent analysis. Yolk constituents analyzed in these trials included moisture, total lipids, cholesterol, triglycerides, phospholipids, and fatty acids. In both trials, total yolk lipid at 22 wk of age was significantly decreased in birds inoculated with FMG. In trial 1, yolk cholesterol at 28 wk was significantly decreased in FMG-inoculated birds. Yolk linoleic acid in trial 1 and yolk stearic and arachidonic acids in trial 2 were significantly increased in FMG-inoculated birds compared to FMG-free birds. In trial 2, yolk myristic, palmitoleic, and oleic acid percentages were significantly decreased in FMG-inoculated birds compared to FMG-free birds. These data suggest that alterations in egg production in commercial layers in response to an FMG infection at 12 wk of age are associated with changes in yolk composition.

(*Key words*: egg, layer, lipid, *Mycoplasma gallisepticum*, yolk)

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INTRODUCTION

Many investigators have suggested that *Mycoplasma gallisepticum* (MG) is present in a high percentage of multiage commercial egg-producing complexes (Carpenter et al., 1981; Mohammed et al., 1986a, 1987; Kleven, 1998). Current treatments for MG may not prevent the disease from spreading but may prevent severe drops in egg production (EP) (Barnes et al., 1960, 1961; Yoder et al., 1961; Cummings et al., 1986; Timms et al., 1989). Live vaccines are effective in minimizing EP loses if administered to commercial layers before exposure to more virulent field strains of MG (Luginbuhl et al., 1976). The most commonly used live vaccine in the United States is F-strain MG (FMG) (Barbour et al., 2000). The prevalent means of live MG vaccine application in a commercial pullet setting is by coarse spray at 8 to 10 wk of age (J. B. Self, 2002, Cal-Maine Foods, Inc., Jackson, MS, personal communication); nevertheless, inoculations with FMG between 8 and 18 wk of age allow a pullet to receive a mild infection and recover before coming into EP (Yoder et al., 1984).

Branton et al. (1997) reported that EP beginning at 22 wk of age (second week of production) and other egg characteristics in birds inoculated with FMG at 10 wk of age were not significantly different from controls. However, in a more recent report using the same birds as in this study being reported, Burnham et al. (2002a) reported that EP and other egg characteristics beginning at onset of lay (18 wk of age) in birds inoculated with FMG at 12 wk of age were significantly different. Burnham et al. (2002a) have reported that initiation of lay was delayed and that EP after 42 wk was reduced in layer hens inoculated with FMG at 12 wk of age. It was also determined

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Abbreviation Key: EP = egg production; FA = fluorescent antibody; FMG = F-strain of *Mycoplasma gallisepticum*; GC = gas chromatography/chromatograph; HI = hemagglutination-inhibition; MG = *Mycoplasma gallisepticum*; MS = *Mycoplasma synoviae*; SPA = serum plate agglutination; YCH = yolk cholesterol; YL = yolk total lipids; YM = yolk moisture; YP = yolk phospholipid; YT = yolk triglycerides.

that FMG-inoculated hens had fewer mature ovarian follicles and decreased magnal, isthmal, and vaginal proportions of the reproductive tract at trial termination (60 wk) when compared to FMG-free hens (Burnham et al., 2002b). The establishment of systemic FMG infections and the long-term changes in reproductive performance and organ characteristics of inoculated birds observed by Burnham et al. (2002a,b) suggest that these changes are a result of the disease itself rather than just a postvaccinal reaction. Previous research has been aimed at determining the effects of FMG on general egg characteristics of commercial egg-laying chickens. However, no literature is available concerning specific possible concomitant alterations in the constituents of egg yolks from laying hens.

Mycoplasma gallisepticum has been cultured from the liver (Sahu and Olson, 1976) and periovarian region (Fabricant and Levine, 1963) of chickens. Burnham et al. (2002b) have reported increased incidences of fatty liver hemorrhagic syndrome in birds having been previously inoculated with FMG at 12 wk of age. Furthermore, other studies comparing egg yolk and serum for the detection of MG antibodies by enzyme-linked immunosorbent assays or hemagglutination inhibition tests showed that egg yolk samples could be used instead of serum samples for flock screening (Yoder and Hopkins, 1985; Mohammed et al., 1986b; Brown et al., 1991). Therefore, colonization of MG in the liver and ovary has the potential of altering yolk composition.

The objectives of this study were to assess yolk characteristics in birds inoculated with FMG at 12 wk of age and describe possible relationships between these effects with those associations in previously reported work concerning the effects of 12-wk inoculations of FMG on reproductive organ characteristics and the performance parameters of laying hens. The yolk constituents described in this study included yolk moisture (YM), total lipids (YL), fatty acids, cholesterol (YCH), triglycerides (YT), and phospholipids (YP).

MATERIALS AND METHODS

Pullet Housing and Management

In each of two trials, one thousand 1-d-old pullets of a single genetic strain were obtained from a commercial source that was monitored and certified free for MG and *M. synoviae* (MS) (National Poultry Improvement Plan and Auxiliary Provisions, 1995). Chicks were vaccinated at 10 d of age for infectious bursal disease via the drinking water. At 12 d and again at 4 wk of age, chicks were also vaccinated for Newcastle Disease and infectious bronchitis by the same route. At 5 wk of age, 10 randomly selected pullets were bled from the left wing vein and tested for antibodies to MG and MS using both the serum plate agglutination (SPA) and the hemagglutination-inhibition (HI) tests (Yoder, 1975). At the same time, swabs were collected from the choanal cleft (Branton et al., 1984) and placed into tubes containing Frey's broth medium (Frey et al., 1968) supplemented with an additional 0.15 mg thallium acetate and 10^6 IU penicillin-G per milliliter. Tubes were incubated at 37°C for 30 d or until a phenol red indicator reaction occurred in the media. A sample from those that changed color was then inoculated onto Frey's-based (Papageorgiou medium) agar and incubated at 37°C. Colonies with morphology suggestive of *Mycoplasma* species were examined by an agar plate fluorescent antibody (FA) method (Baas and Jasper, 1972) that used direct labeling of colonies stained with anti-FMG polyclonal antibodies produced in rabbits and labeled with fluorescein isothiocyanate (Kleven, 1981).

Until the pullets were 12 wk of age, they were placed on clean dry litter in a 5.5×6.1 m section of a conventional house resulting in an initial flock density of 0.034 m^2/bird. A daily artificial lighting schedule followed a 13L:11D cycle. One 75-W incandescent light bulb was used to illuminate each 8.4 m^2 of floor space, providing a calculated intensity at bird level of 35.5 lx. Feed and water were provided ad libitum in each trial. Ingredient percentages and dietary analyses of the basal starter and grower diets used in both trials were reported by Burnham et al. (2002a). All diets were formulated to meet or exceed National Research Council (1994) specifications. Because birds were placed on clean litter, no anticoccidials were added to the feed. No medication was administered during the interval of either trial.

At 12 wk of age, 11 pullets were randomly selected and placed in each of eight (trial 1; total of 88 pullets) or 16 (trial 2; total of 176 pullets) negative pressure fiberglass biological isolation units (1.16 m^2). The units were housed in a previously described poultry disease isolation facility (Branton and Simmons, 1992). Ingredient percentages and dietary analyses of the basal developer and prelay diets used in both trials were reported by Burnham et al. (2002a).

Layer Housing and Management

Hen numbers were reduced to 10 per unit at point-of-lay (18 wk of age) so that bird density was 0.116 m^2/bird for the duration of each trial. In each trial, half of the total number of isolation units contained FMG-free control birds, whereas the other half contained FMG-inoculated birds. There were four replicate units per treatment in trial 1 and eight replicate units per treatment in trial 2. Beginning at 18 wk of age, the artificial lighting schedule was increased 15 min/d until a 16 h 15 min L:7 h 45 min D cycle was achieved. Chickens were maintained on that schedule through the remainder of the experiments. Ingredient percentages and dietary analyses of the layer diets used in both trials were reported by Burnham et al. (2002a). In both trials at 26 and 54 wk of age, quadruplicate feed samples per lot of mixed feed were analyzed for moisture, ash, CP, crude fat, and crude fiber. All determined analyses were performed according to the methods of the Association of Official Analytical Chemists (1980) and averaged for each of the two trials at each time period. Available protein and lysine percentages in the layer diet were adjusted according to the percentage of feed con-

sumed per bird every 28 d until trial termination (54 wk in trial 1 and 60 wk in trial 2).

FMG Inoculation

In each trial, pullets treated with FMG were inoculated via eye drop in the right eye at 12 wk of age with 0.04 mL of a 24-h broth culture of high-passage FMG (99th passage above the unknown passage level) provided by S. H. Kleven.[4] Inoculum titers were 5.0×10^6 and 1.0×10^5 cfu/mL in trials 1 and 2, respectively. Similarly, pullets designated as controls were sham-inoculated via eye drop in the right eye at 12 wk of age with 0.04 mL of sterile Frey's broth medium. Inoculum volume, titer levels, and mode of administration were as previously specified by Branton and Deaton (1985) and achieve an efficacy of inoculation comparable to that in the commercial industry.

Mycoplasma Detection

In each trial at 20 wk, and again at 54 wk in trial 1 and 58 wk of age in trial 2, one randomly selected hen from each of four FMG-free control and FMG-treated isolation units was bled and swabbed. Each of these samples was tested for the presence of *Mycoplasma* species as previously described for pullets.

Data Collection

Eggs were collected, and yolks were harvested several times during the prepeak, peak, and postpeak periods of both trials for constituent analysis. More specifically, a pool of 10 egg yolks per replication from control and treatment groups was harvested at 22, 24, 28, 30, 32, 36, 40, 44, 48, and 52 wk of age in trial 1 and at 22, 24, 28, 34, 40, 46, 52, and 58 wk of age in trial 2.

Quantitation of YM and YL

For analysis of YM content, duplicate fresh yolk samples (2 g) were dried according to the procedure of Peebles et al. (1999) in a commercial oven.[5] Yolk moisture contents were calculated as the difference between their wet and dry weights and were expressed as a percentage of wet yolk sample weight. For analysis of YL content, lipid was extracted from duplicate fresh yolk samples (3 g) according to the procedure previously described by Bligh and Dryer (1959) and as modified by Latour et al. (1998). Total YL was expressed as a percentage of fresh yolk sample weight. The YL was dissolved in 2 mL of hexane, 200 μL of 0.83% butylated-hydroxy toluene, and refrigerated, as described by Christie (1982) for further content analyses as described below.

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[5]Model EL20, General Electric Co., Chicago Heights, IL.
[6]Lab-Line Instruments, Inc., Melrose Park, IL.
[7]Hewlett Packard Co., Boise, ID.
[8]Waters Corp., Milford, MA.

Methyl Esterification of Yolk Lipids

Duplicate lipid samples were methylated according to the procedure described by Morrison and Smith (1964). A Multi-Block[6] system was used to boil each sample in a test tube at 80 ± 0.5°C for 30 min. A 200 μL aliquot of the solution was placed in a 2-mL gas chromatography (GC) vial along with 400 μL of isooctane and sealed with a rubber-lined cap for further fatty acid analyses by GC as described below.

Chromatographic Analysis of Yolk Contents

Fatty acid profiles of duplicate YL samples were determined at 24, 28, 32, 36, 40, and 44 wk of age in trial 1 and at 24, 34, 40, 46, 52, and 58 wk of age in trial 2 with a 5890 A, Series I GC[7] according to the procedure by Latour et al. (1998). Fatty acids were identified by comparing peak retention times against polyunsaturated fatty acids and rapeseed oil. The standards were injected periodically to ensure accurate measurement by the GC. The individual fatty acids retained by the GC were expressed as a percentage of the total fatty acid content of the fresh yolk sample. Determination of YCH was performed at 24, 28, 32, 36, 40, and 44 wk of age in trial 1 and at 22, 24, 28, 34, 40, 46, 52, and 58 wk of age in trial 2 by direct saponification followed by a procedure that utilized capillary liquid GC (Maurice et al., 1994). Fresh yolks were used to determine YCH in milligrams per gram of total yolk.

YP and YT Analysis

For determination of YP and YT, lipids were previously extracted by the method described by Bligh and Dryer (1959) and Folch et al. (1957). Rapid, efficient, high recovery Sep-Pak[8] cartridges were used to separate and isolate polar and neutral/nonpolar lipids (Hamilton and Comai, 1984, 1988; Figewicz et al., 1985; Juaneda and Rocquelin, 1985: Kaluzny et al., 1985) at 24, 28, 32, 36, 40, and 44 wk of age in trial 1. Nonpolar lipids were eluted with 20 mL of chloroform to collect triglycerides, followed by 20 mL of chloroform/methanol (49:1, vol/vol) to discard monoacyl and diacyl glycerides. Polar lipids [primarily (98%) composed of YP (Hamilton and Comai, 1988)] were then eluted with 30 mL of methanol, all with a flow rate of 25 mL/min. After evaporation using a rotary evaporator at 40°C, the respective weights of YT and YP were determined with an electronic balance, and their concentrations were quantified as percentages of total YL.

Statistical Analysis

A completely randomized experimental design was utilized. Egg YM, YL, fatty acids, YCH, YT, and YP were subjected to a repeated measures analysis where the same experimental units were observed over multiple age periods. Individual sample data within each replicate unit

were averaged prior to analysis. Least-squares means were compared in the event of significant global effects (Steel and Torrie, 1980; Petersen, 1985; Freund and Wilson, 1997). All data were analyzed using the MIXED procedure of SAS, Version 8 (1996). Statements of significance were based on $P \leq 0.05$ unless otherwise stated.

RESULTS

In both trials, all initial mycoplasmal cultures, as well as SPA and HI test results obtained from 5-wk-old pullets, were negative for MG and MS. Control serum samples obtained at 20 wk of age in each trial and also at 54 wk (trial 1) and 58 wk (trial 2) were SPA and HI negative for MG, while the same tests were positive for MG in the FMG-inoculated hens. Hens were considered FMG-free when they exhibited no detectable HI titers. All FMG-inoculated hens had HI titers ≥ 1:80. Similarly, FA culture results for swabs obtained at 20 wk of age in each trial and also at 54 wk (trial 1) and 58 wk (trial 2) were negative for *Mycoplasma* species growth for four out of four FMG-free hens tested, while growth was evident for four out of four FMG-inoculated hens tested.

Yolk moisture in both trials and YCH content in trial 2 changed ($P \leq 0.0001$) with bird age. Yolk moisture was highest at 22 wk in both trials and lowest at 52 wk in trial 1 and at 58 wk in trial 2. Yolk cholesterol was highest at 22 wk and lowest at 58 wk in trial 2. In both trials, yolk myristic ($P \leq 0.03$), palmitic ($P \leq 0.0001$), stearic ($P \leq 0.0003$), palmitoleic ($P \leq 0.006$), oleic ($P \leq 0.01$), linoleic ($P \leq 0.0004$), linolenic ($P \leq 0.01$), and arachidonic ($P \leq 0.05$) acid concentrations changed with bird age. Nonessential fatty acid concentrations, which included myristic, palmitic, and stearic acids, were lowest and essential fatty acid concentrations, which included palmitoleic, oleic, linoleic, linolenic, and arachidonic acids, were highest at 24 wk in both trials. However, at 44 wk in trial 1 and at 58 wk in trial 2 nonessential fatty acid concentrations were highest and essential fatty acid concentrations were lowest.

There were age by FMG treatment interactions for YL in trial 1 ($P \leq 0.02$) and in trial 2 ($P \leq 0.0001$) (Table 1). In both trials, when compared to controls, YL from FMG-treated hens was significantly lower at 22 wk. Also, YL from FMG-treated hens was significantly higher at 32 and 44 wk and significantly lower at 48 wk of age in trial 1. There was an age by FMG treatment interaction ($P \leq 0.02$) in trial 1 for YCH concentration (Table 2). Concentration of YCH in trial 1 was significantly decreased at 28 wk of age in birds infected with FMG compared to FMG-free controls.

There were main effects due to FMG-inoculation for the percentage of yolk linoleic acid ($P \leq 0.04$) in trial 1 and stearic ($P \leq 0.03$), arachidonic ($P \leq 0.01$), myristic ($P \leq 0.03$), palmitoleic ($P \leq 0.05$), and oleic ($P \leq 0.01$) acid concentrations in trial 2 (Table 3). Linoleic, stearic, and arachidonic acid concentrations were significantly increased, while myristic, palmitoleic, and oleic acid concentrations were significantly decreased in FMG-inoculated birds compared to FMG-free birds.

DISCUSSION

At the beginning and end of both trials in this study, SPA tests from swabs and sera, HI sera tests, and FA tests, verified systemic infections in FMG-inoculated birds. Conversely, sham-inoculated birds remained FMG-free throughout each trial. *Mycoplasma gallisepticum* has been cultured from the liver (Sahu and Olson, 1976) and periovarian region (Fabricant and Levine, 1963) of chickens. Furthermore, egg yolks can be used for the detection of MG infections in birds (Yoder and Hopkins, 1985; Mohammed et al., 1986b; Brown et al., 1991). Although it is known that circulating triglyceride levels increase in response to infections by pathogens (Funder and Sheppard, 1987; Guyton and Hall, 1996), no other information is available concerning possible alterations in yolk content after an MG challenge. A significant decrease in YL at 22 wk in FMG-infected hens suggests that FMG may inhibit YL deposition at that time. Increased percentages of YL postpeak, however, may represent a compensatory increase in YL deposition in response to the earlier depression in YL. Furthermore, decreases in YCH at 28 wk (prepeak) due to FMG-infection support the conclusion that FMG may interfere with the deposition of YL in young layers.

Because specific lipid profiles, including those of fatty acids in the blood of layer hens inoculated with FMG at 12 wk of age, have never been determined, it is not possible to ascertain whether or not changes in yolk characteristics of FMG-infected hens are due to alterations in the liver only or in both the liver and ovary. However, because enzymatic activity in follicles destined to become yolks can change drastically during reproduction (Chalana and Guraya, 1978), it is possible that changes in yolk characteristics may involve FMG colonization of the ovary. However, changes in YL deposition are likely to occur through colonization of the liver. Formation of yolk protein and lipid mainly occur in the liver (Johnson, 1986, 2000). A chicken's liver is also responsible for synthesis of fatty acids de novo (O'Hea and Leveille, 1969; Donaldson, 1990). Mammalian and avian livers are rich in lipid and the process of fatty acid synthesis in each have many common features (Poulose et al., 1981; Naggert et al., 1988). The distribution of fatty acids of various chain lengths is relatively constant in YL and is related to their synthesis in the hens' liver (Watkins and Kratzer, 1987; Watkins, 1995; Walzem, 1996; Speake and Thompson, 1999). Because MG may be cultured from the livers of infected chickens (Sahu and Olson, 1976), liver colonization by MG may play a role in the changes in YL profiles of infected birds.

In the current study, yolk fatty acid composition was altered by inoculation with FMG at 12 wk of age. Altered fatty acid profiles of yolks from MG-inoculated hens may be due more specifically to altered activities of various liver lipid enzymes. The conversion of palmitate to palmi-

TABLE 1. Yolk lipid in F-stain *Mycoplasma gallisepticum* (FMG)-free and FMG-inoculated Single Comb White Leghorn laying hens at 22, 24, 28, 30, 32, 34, 36, 40, 44, 46, 48, 52, and 58 wk of age in trials 1 and 2

	Trial 1[1]		Trial 2[1]	
Age (wk)	FMG-free	FMG-inoculated	FMG-free	FMG-inoculated
	(% of total yolk)			
22	27.6[a,2]	26.7[b]	26.5[a]	13.1[b]
24	27.0	27.2	28.4	28.9
28	26.0	26.1	28.1	28.4
30	26.1	26.4	ND	ND
32	27.3[b]	28.5[a]	ND	ND
34	ND[3]	ND	29.2	28.7
36	28.7	28.3	ND	ND
40	29.0	28.3	30.1	29.2
44	28.4[b]	29.8[a]	ND	ND
46	ND	ND	28.2	28.5
48	30.2[a]	29.2[b]	ND	ND
52	29.4	28.7	30.4	30.4
58	ND	ND	29.2	29.4

[a,b]Means within trial among week of age and treatment group with no common superscript differ significantly ($P \leq 0.05$).

[1]Based on pooled estimate of variance SEM = 0.37 and 0.99 in trials 1 and 2, respectively.

[2]n = 4 samples for the calculation of means within treatment and week.

[3]Not determined.

toleate occurs when a double bond is introduced into the fatty acid chain by an oxidative reaction catalyzed by Δ9-fatty acyl-CoA desaturase (Lehninger, 1975; Cook, 1991). Palmitate can also be elongated by further additions of acetyl groups from the smooth endoplasmic reticulum and mitochondria to form stearoyl coenzyme A (stearate), which can further be desaturated by Δ9-desaturase to produce oleate (Cook, 1991). These data suggest that there is a shift in the elongation process from palmitic to stearic acid instead of the Δ9-fatty acyl-CoA desaturase process to form palmitoleic acid in the livers of FMG-inoculated birds. Also, it is possible that Δ9-desaturase activity is depressed to allow for the build up of stearic acid, while oleic acid concentration is depleted. Ding and Lilburn (1996) reported that oleic acid comprised the largest proportion of total yolk fatty acids. Although linoleate, an essential fatty acid, cannot be synthesized from oleate in animals, once ingested, linolenic acid is derived from linoleic acid through appreciable Δ6-desaturation activity (Noble and Shand, 1985; Noble and Cocchi, 1990). Linolenic acid is elongated to form eicosatrienoic acid, and subsequently, arachidonic acid is derived from eicosatrienoate acid through appreciable Δ5-desaturation activity (Lehninger, 1975; Cook, 1991). Increased yolk arachidonic acid concentrations may have ultimately resulted from increased liver Δ5-desaturase activity. Evans et al. (1962) reported that dietary cottonseed oil supplementation increased the percentage of stearic acid and decreased the percentage of oleic acid in the yolks of layer hen eggs. Raju and Reiser (1967) showed that the

TABLE 2. Yolk cholesterol in F-strain *Mycoplasma gallisepticum* (FMG)-free and FMG-inoculated Single Comb White Leghorn laying hens at 22, 24, 28, 32, 34, 36, 40, 44, 46, 52, and 58 wk of age in trials 1 and 2

	Trial 1[1]		Trial 2[1]	
Age (wk)	FMG-free	FMG-inoculated	FMG-free	FMG-inoculated
	(mg/g)			
22	ND[3]	ND	13.5	13.4
24	12.1[2]	12.8	13.1	12.9
28	13.5[a]	12.2[b]	13.1	13.0
32	12.2	12.8	ND	ND
34	ND	ND	12.7	12.9
36	12.6	12.4	ND	ND
40	11.4	11.7	12.3	12.0
44	11.8	12.2	ND	ND
46	ND	ND	12.0	12.1
52	ND	ND	12.0	11.9
58	ND	ND	11.9	12.1

[a,b]Means within trial among week of age and treatment groups with no common superscript differ significantly ($P \leq 0.05$).

[1]Based on pooled estimate of variance SEM = 0.54 and 0.23 in trials 1 and 2, respectively.

[2]n = 4 samples for the calculation of means within treatment and week.

[3]Not determined.

TABLE 3. Yolk myristic, palmitic, stearic, palmitoleic, oleic, linoleic, linolenic, and arachidonic acids in F-strain *Mycoplasma gallisepticum* (FMG)-free and FMG-inoculated Single Comb White leghorn laying hens in trials 1 and 2

	Trial 1[1]		Trial 2[2]	
Fatty acid	FMG-free[3]	FMG-inoculated[3]	FMG-free[4]	FMG-inoculated[4]
	(% of total fatty acids)			
Myristic	0.3	0.3	0.3[a]	0.2[b]
Palmitic	27.9	27.9	27.8	28.1
Stearic	12.2	12.0	11.7[b]	12.3[a]
Palmitoleic	2.3	2.3	2.2[a]	2.0[b]
Oleic	32.9	33.0	34.5[a]	32.9[b]
Linoleic	13.0[b]	13.5[a]	12.7	12.7
Linolenic	0.4	0.4	0.3	0.3
Arachidonic	4.6	4.3	4.2[b]	4.8[a]
Other	6.6	6.3	6.3	6.6

[a,b]Means within trial and fatty acid among treatment groups with no common superscript differ significantly ($P \leq 0.05$).

[1]Based on pooled estimate of variance SEM for myristic, palmitic, palmitoleic, stearic, oleic, linoleic, linolenic, and arachidonic fatty acids in trial 1 = 0.01, 0.18, 0.07, 0.16, 0.41, 0.12, 0.01, and 0.18.

[2]Based on pooled estimate of variance SEM for myristic, palmitic, palmitoleic, stearic, oleic, linoleic, linolenic, and arachidonic fatty acids in trial 2 = 0.01, 0.14, 0.06, 0.15, 0.29, 0.11, 0.02, and 0.13.

[3]n = 24.

[4]n = 32.

cyclopropenoid fatty acids (constituents of crude cottonseed oil) inhibited a fatty acyl desaturase, which normally converts stearic acid to oleic acid. Alterations in fatty acid precursors or fatty acyl desaturase activities may yield variations in the formation of subsequent fatty acids; the degree or type of MG colonization in the liver might also lead to changes in fatty acid metabolites due to conformational changes in liver fatty acid precursors and enzymes.

In the current study, inoculation with FMG at 12 wk of age did not significantly affect the concentrations of YT and YP within the egg yolk. Since YT and YP concentrations are similar in FMG-free control and FMG-treated birds, it appears that quantity of total lipid and the types of fatty acids deposited in yolk can change without concomitant changes in YT and YP due to an FMG infection.

Burnham et al. (2002a) have reported that initiation of lay was delayed and that weekly EP after 42 wk and overall average weekly EP were reduced in layer hens inoculated with FMG at 12 wk of age. Inoculation with FMG at 12 wk altered lipid concentrations in blood (Burnham et al., 2000), which may lead to decreases in YL concentration at the prepeak period. This, in turn, may have resulted in a delay in onset of lay and a reduction in EP. Palmer and Bahr (1992) have suggested that increased follicular atresia and reduced follicle numbers during the final 6 to 11 d prior to ovulation can result in decreased EP. It has also been reported (Burnham et al., 2002b) that FMG-inoculated layer hens possessed fewer mature follicles when compared to FMG-free hens. Colonization of FMG within the ovary probably does not affect YL, including YT, YP, and fatty acid distribution but may alter the ovulatory process. These data suggest that F-strain MG may be affecting EP, as noted in a previous report, through changes in yolk deposition and rates of follicle formation and ovulation. It is concluded that FMG colonization in the liver of laying hens significantly affects EP through alterations in YL concentrations and also affects the metabolism and production of various fatty acids that are ultimately deposited in the yolk.

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